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Research Note

Genetic diversity and population structure of *Brachiaria brizantha* (A.Rich.) Stapf accessions from Ethiopia

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Brachiaria **is a tropical, warm-season grass native to Africa. It is an extensively cultivated forage in the tropics with proven benefits on livestock productivity.** ***Brachiaria*** **is well-known for high biomass production, animal nutrition, carbon sequestration, biological nitrification inhibition, soil conservation, and adaptation to drought and low fertility soils. However, the use of** ***Brachiaria*** **grass for fodder production in Africa has been little explored largely due to lack of cultivars suitable to different production environments. The exploration and use of natural diversity is fundamental for an efficient** ***Brachiaria*** **breeding program. We analysed genetic diversity and population structure of 112 Ethiopian** ***Brachiaria brizantha*** **accessions using 23 microsatellite markers. A total of 459 alleles were detected with an average polymorphic information content of 0.75 suggesting high discriminating ability of these markers. The molecular variance analysis showed a high contribution (86%) of within-cluster differences to the total variation. Three allelic pools revealed by STRUCTURE analysis in 112 accessions were in agreement with the clustering patterns seen in neighbor-joining tree and principal coordinates analyses. A core collection of 39** ***B. brizantha*** **accessions was constituted. This study concludes a high genetic diversity of Ethiopian** ***B. brizantha*** **accessions and their importance in** ***Brachiaria*** **breeding programs.**

Keywords: accessions, *Brachiaria brizantha*, core collection, genetic diversity, population structure

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Brachiaria (Trin.) Griseb., consisting of over 100 species, is distributed across the tropics, particularly tropical Africa (Renvoize et al. 1996). Seven *Brachiaria* species of African origins – *B. arrecta*, *B. brizantha*, *B. decumbens*, *B. dictyoneura*, *B. humidocila*, *B. mutica* and *B. ruziziensis* –are used for pasture production (Keller-Grein et al. 1996). *Brachiaria* produces a high amount of quality biomass and improves livestock productivity (Reid et al. 1973; Jotee 1988; Holmann et al. 2004). Moreover, *Brachiaria* is adapted to drought and low-fertility soils, sequesters carbon, enhances nitrogen use efficiency, and subsequently minimises eutrophication and greenhouse gas emission (Subbarao et al. 2009; Arango et al. 2014; Moreta et al. 2014). *Brachiaria* is the most extensively cultivated tropical forage in monoculture supporting millions of livestock across the globe with an acreage of over 99 million ha in Brazil alone (Jank et al. 2014).

The International Center for Tropical Agriculture (CIAT) and Brazilian Agricultural Research Corporation (EMBRAPA) developed some improved *Brachiaria* cultivars (Argel and Keller-Grein 1996) for the tropical Americas. Evaluations of eight improved *Brachiaria* cultivars in Kenya revealed that most of them showed good adaptation, high biomass production and increased livestock productivity but showed susceptibility to different diseases (Njarui et al. 2016). Moreover, recent studies have found a narrow genetic base of these improved cultivars with a high genetic similarity (Ondabu et al. 2017; Kuwi et al. 2018). These observations raise a substantial risk of increasing acreage of these improved cultivars in Africa.

Eastern Africa represents a centre of diversity of the genus *Brachiaria*, and *B. brizantha* occurs naturally in Ethiopia (Keller-Grein et al. 1996). Thus, an assessment of genetic diversity of *B. brizantha* should be conducted to establish an effective *Brachiaria* breeding program in Ethiopia. In this study, we analysed the genetic diversity and population structure of 112 Ethiopian *B. brizantha* accessions from the Forage Field Genebank of the International Livestock Research Institute (ILRI), Ethiopia, examined the genetic relatedness of the tested accessions and highlight implications of these findings for *Brachiaria* breeding programs.

We studied 112 Ethiopian *B. brizantha* accessions from the ILRI Forage Field Genebank, Zwai, Ethiopia (Supplementary

Table 1) and six improved cultivars – *B. brizantha* 'MG4', *B. brizantha* 'Piatã', *B. decumbens* 'Basilisk', *B. humidicola* 'Humidicola', *B. humidicola* 'Llanero' and the hybrid cultivar 'Mulato II'. Genomic DNA was extracted from young healthy leaves using the ZR plant/seed DNA MiniPrep™ Kit following the manufacturer's instructions (Zymo Research, Irvine, CA, USA). The DNA concentration, purity and integrity were determined as described previously (Kuwi et al. 2018).

Twenty-three simple sequence repeat (SSR) primers initially developed for *B. ruziziensis* with proven transferability to other *Brachiaria* species were used (Silva et al. 2013; Supplementary Table 2). Primer optimisation, gel electrophoresis, multiplex PCR and capillary electrophoresis were performed as described previously (Kuwi et al. 2018). Allele calling and sizing were performed manually using GeneMapper 4.1 software (Applied Biosystems, Foster City, CA, USA). Due to the polyploid nature of *B. brizantha*, SSRs were scored as dominant markers (Jungmann et al. 2010; Vigna et al. 2011). Both allelic and binary data were used to assess genetic diversity.

The Bayesian model-based clustering algorithm was implemented in STRUCTURE 2.3.4 software to infer population structure (Pritchard et al. 2000). To estimate the posterior probabilities (qK) a 150 000 burn-in period was used, followed by 300 000 iterations using a model allowing for admixture and correlated allele frequencies with no *a priori* location and population information. A batch job with values of *K* ranging from 1 to 10 was set, with 20 independent runs for each *K* setting and other parameters at default values. The ΔK was calculated for each value of *K* using Structure Harvester (Evanno et al. 2005; Earl and vonHoldt 2011). Accessions were included in a cluster when a *Q* value for any cluster was ≥0.60. Bar plots were generated with average results of runs for the most probable *K* value using Distruct 1.1 (Rosenberg 2003).

Pairwise genetic dissimilarity matrices were calculated using Dice's similarity coefficient, and a dendrogram was constructed (for ecotypes and six improved cultivars) using the unweighted neighbour-joining (UNJ) method. The bootstrap calculation was performed based on 1 000 replications in DARwin 6.0 (Perrier and Jacquemoud-Collet 2006). Roger's genetic distance was used to construct the pairwise genetic distance between each pair of accessions. Principal coordinate analysis (PCoA) and Analysis of Molecular Variance (AMOVA) were performed using GenAlEx 6.5 (Peakall and Smouse 2012). A genetic core collection was assembled using the Min SD subset algorithm procedure in DARwin 6.0 (Perrier and Jacquemoud-Collet 2006).

Twenty-three SSR markers detected 459 alleles with an average of 4.9 alleles per locus (Supplementary Table 2). The polymorphic information content (PIC) ranged from 0.32 to 0.94 and Br0130, Br0149 and Br0235 were the three most polymorphic loci with the highest PIC values. The STRUCTURE analysis combined with Evanno ΔK statistics revealed 3 distinct gene pools ($K = 3$) and minor peaks at $K = 5$. The clusters 1, 2 and 3 were represented by 26, 39 and 47 accessions, respectively (Figure 1). Five accessions from cluster 2 and four accessions from cluster 3 were an admixture.

The relationships among accessions were visualised in the UNJ tree (Figure 2). To compare the grouping of accessions in the UNJ and STRUCTURE analyses, the branches of the tree were coloured according to STRUCTURE simulations for preset $\Delta K = 3$ (clusters 1–3). Accessions (except a few) from cluster 1 grouped together in the UNJ tree. Similarly, most accessions from cluster 2 grouped together in the UNJ tree. Only 17 accessions from cluster 3 were grouped together in the UNJ tree, whereas the remainder were placed in different groups. All six improved cultivars grouped together in the UNJ tree. The genetic distance among most pairs of individuals clearly showed a high level of variation, which ranged from 0.6 to 0.90 (Supplementary Figure 1).

The PCoA well supported the UNJ tree and STUCTURE analysis. The first two coordinates explained 24% of the total variation and grouped 112 *B. brizantha* accessions in three major clusters (Figure 3). The accessions that formed cluster 1 in the STRUCTURE analysis (green) were also observed in a single cluster in the PCoA. Similarly, most cluster 2 accessions in the STRUCTURE analysis (red)

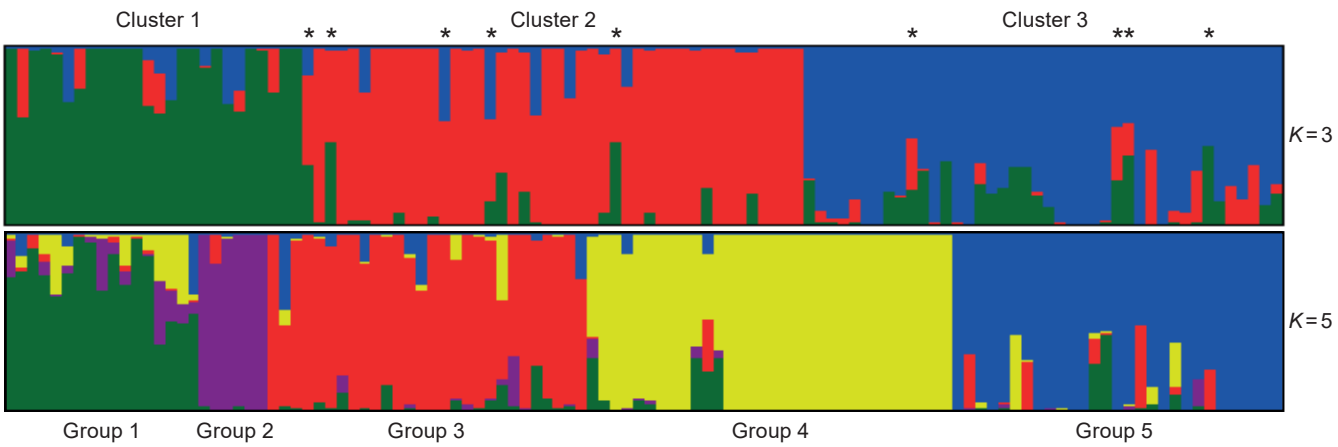

Figure 1: STRUCTURE bar plot of membership coefficients for 112 *Brachiaria brizantha* accessions sorted in the same order and classified according to selected *K* values of 3 and 5. Accessions were allocated to gene pools or clusters based on *Q* values ($Q > 0.6$) and addmixed accessions are identified with an asterisk (*) in $\Delta K = 3$

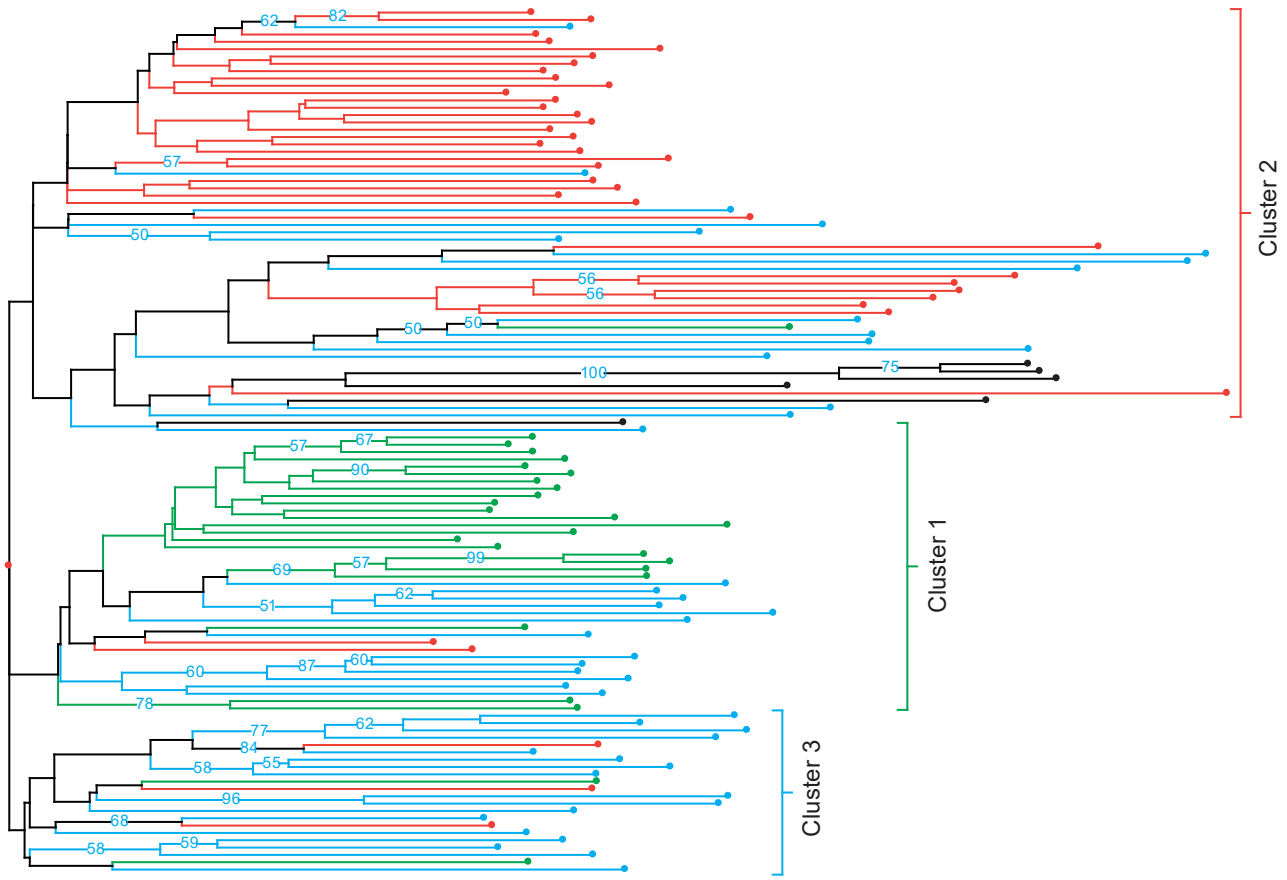

Figure 2: Unweighted neighbour-joining tree constructed using Dice's coefficient based on 23 microsatellite loci for 112 Ethiopian *Brachiaria brizantha* accessions and six improved cultivars. Branches are coloured according to the structure simulation for $K = 3$; improved cultivars are highlighted in black

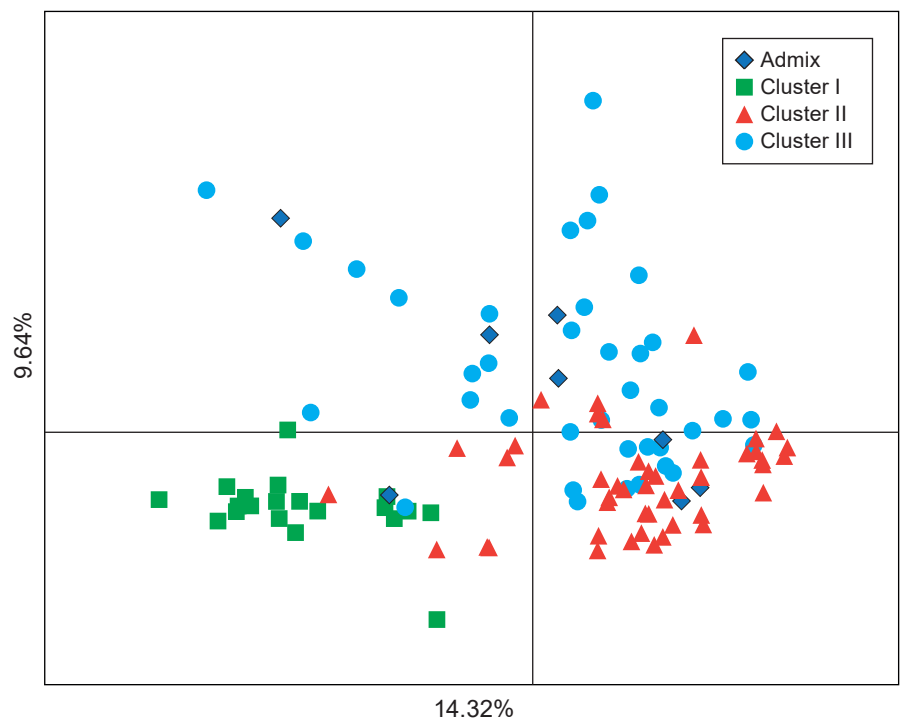

Figure 3: Principal coordinates analysis bi-plot showing the clustering of the 112 Ethiopian *Brachiaria brizantha* accessions

formed a group in the PCoA. However, cluster 3 accessions (blue) were somewhat dispersed.

A hierarchical partitioning of genetic variation showed a significant ($p < 0.001$) variation among clusters. The analysis indicated that 86% of the total variation was contributed by within-cluster differences (Supplementary Table 3). A genetic core of 39 accessions captured all 459 alleles detected in the 112 *B. brizantha* accessions (Supplementary Table 4).

The microsatellite markers used in this study were highly informative and well-differentiated Ethiopian *B. brizantha* accessions. The average PIC value for these markers (0.75) was higher than that in most previous studies (Jungmann et al. 2009a; Vigna et al. 2011; Silva et al. 2013; Ondabu et al. 2017; Kuwi et al. 2018). Similarly, the average number of alleles per locus (19.90) was higher than that in previous studies (7.70 to 16.96) (Jungmann et al. 2009b; Vigna et al. 2011; Pessoa-Filho et al. 2015; Kuwi et al. 2018). The high number of alleles in this study was attributed to multiple factors, including diverse geographical origins of accessions, and the use of markers with high PIC values and transferability to other *Brachiaria* species.

This study reports the high contribution of within-cluster differences (86%) to total variation in Ethiopian *B. brizantha* accessions. This result was in agreement with other studies on *B. brizantha* and *B. ruziziensis* (Vigna et al. 2011; Pessoa-Filho et al. 2015), but was higher than that in a study on *B. humidocola* (Jungmann et al. 2010). Such variable results are likely due to polyploidy, apomictic reproduction and occurrence of natural hybridisation in *Brachiaria* species (do Valle and Savidan 1996; Jungmann et al. 2010).

The STRUCTURE analysis grouped 112 *B. brizantha* accessions into three clusters suggesting scope for crossing individuals from different clusters to explore heterosis (Vigna et al. 2011). The clustering of the accessions was independent of their geographical origins as reported in other studies on *B. brizantha* and *B. humidocola* accessions (Jungmann et al. 2010; Vigna et al. 2011). Interestingly, some accessions from cluster 3 of the STRUCTURE bar plot (Figure 1) were dispersed in the UNJ and PCoA analyses which highlighted their genetic differences. Polyploidy, apomictic reproduction and high morphological variation in *B. brizantha* could be causes for such genetic dissimilarity (do Valle and Savidan 1996; Renvoize et al. 1996; Vigna et al. 2011).

The Ethiopian *B. brizantha* accessions analysed in this study showed higher genetic diversity than the six improved cultivars representing four species (*B. brizantha*, *B. decumbens*, *B. humidicola* and *B. ruziziensis*; Figure 2). Similar results were described for Kenyan ecotypes and Tanzania accessions (Ondabu et al. 2017; Kuwi et al. 2018). The presence of three allelic pools and high genetic diversity among Ethiopian *B. brizantha* accessions determines the importance of this germplasm in a *Brachiaria* breeding program. The core collection of 39 accessions constituted in this study will be a valuable resource for *Brachiaria* breeding and conservation programs.

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